that a peptide sequence between the heavy and light chains (or portions thereof) links the two chains together as a single polypeptide. The linker polypeptide is between the two chains regardless of which chain is at the N-terminus of the polypeptide. A single chain Fv fragment is a generally understood example of a single polypeptide immunoglobulin.

Claim 83 has been amended to delete reference to expressing a light chain polypeptide.[1] Claim 83 is directed to a plant comprising plant cells containing an immunoglobulin heavy chain polypeptide, wherein the nucleotide sequence encodes a leader sequence forming a secretion signal for the heavy chain polypeptide product. In this claim the heavy chain is from an antigen-specific immunoglobulin comprising a heavy and light chain, and the single polypeptide product is capable of forming an antigen-specific immunoglobulin when co-expressed in the same cell with the light chain of the antigen-specific immunoglobulin. Also required is that the cells contain immunoglobulin single polypeptide product encoded by the nucleotide sequences, wherein the leader sequence is cleaved from the polypeptide product following proteolytic processing. Claim 83 is intended to cover transgenic plants which separately express the heavy chain of an antigen-specific immunoglobulin. As described in the instant application, such a plant can be crossed with, for example, a light chain expressing plant to yield a plant with plant cells that express both the heavy and light chains which assemble in the plant cell to form an antigen specific immunoglobulin.

Amendment Support

Claim 43 has been amended to recite that nucleic acid which encodes the single polypeptide immunoglobulin encodes the heavy chain polypeptide, the light chain polypeptide and "a peptide linker therebetween." Support for the amendment can be found throughout the specification, in particular to page 10, lines 5-10, which defines a single chain antigen-binding protein and encoding gene.

> A polypeptide composed of an immunoglobulin light-chain variable region amino acid sequence (V_L) tethered to an immunoglobulin heavy-chain variable region amino acid

[1] Applicants intend to pursu the cancelled subject matter in related copending application serial no. 09/512,736.

> sequence (V_H) by a peptide that links the carboxyl terminus of the V_L sequence to the amino terminus of the V_H sequence.");

Also, page 28 lines 5-13, which identifies useful immunoglobulin products in transgenic plants, refers to single chain antigen binding proteins, the structures of which have "been described by Bird et al., Science, 242: 423-426 (1988) and U.S. Patent No. 4,704,692 to Ladner". Accordingly, no new matter is introduced by this amendment.

The amendments to claim 83, which merely deletes reference to expressing a single polypeptide comprising a light chain, finds ample basis in the application. For example, support can be found at Page 64 line 18 ("Example 3") to page 65, line 5, describing preparation of a plant expression vector encoding an immunoglobulin heavy chain single polypeptide with a leader sequence ("The resulting gamma heavy chain expression vector contained a gene coding for the entire gamma heavy chain including the gamma leader."). In addition, see page 68, Table 3 and accompanying text, describing immunological detection of gamma heavy chain expressed as a single polypeptide in a plant. (see numbers under headings "Gamma-L"); Page 75, lines 5-13, describing detection of RNA encoding gamma heavy chain expressed as a single polypeptide in a plant (referring to gamma with leader represented by lanes 3 and 4, respectively, in Figure 4 of U.S. 5,202,422); and Page 76, lines 22-32, describing immunological detection through Western blotting of gamma heavy chain expressed as a single polypeptide in a plant (referring to gamma with leader represented by lanes 5 and 6, respectively, in Figure 5 of U.S. 5,202,422). Accordingly, no new matter is introduced by this amendment.

Support for "portion thereof" in Amendment filed March 4, 2002

Applicants wish to add further comment to the remarks supporting amendment to "portion thereof" in claims 43 and 83 that appeared in the Amendment mailed March 4, 2002. It is respectfully submitted that there is ample support in the specification for this phrase/ As stated previously, support for this phrase is found, for example, at page 10, line 27-33 (emphasis added).

> Immunoglobulin product: **A polypeptide, protein or multimeric protein containing at least the immunologically activ p rtion of an immun globulin heavy chain and is thus capable of specifically combining with an antig n.** Exemplary immunoglobulin products are an immunoglobulin heavy chain,

> immunoglobulin molecules, substantially intact immunoglobulin molecules, **any portion of an immunoglobulin** that contains the paratope, including those portions known in the art as Fab fragments, Fab' fragment, $F(ab')_2$ fragment and Fv fragment.

Reference in the above quote to "at least the immunologically active portion" . . . any portion of an immunoglobulin . . . including those portions known in the art" is broad language strongly demonstrating that any and all fragments were contemplated limited only by the requirement that the fragment be "immunologically active." This would include nearly complete heavy or light chains with only one or a few amino acids removed to deletion mutants lacking particular segments or domains (e.g., a constant region domain). This view is additionally supported by page 3, lines 1-6 (emphasis added) of the specification.

> One of the most useful aspects of using a recombinant expression system for antibody production is the ease with which the antibody can be tailored by molecular engineering. **This allows the production of antibody fragments and single-chain molecules, as well as the manipulation of full-length antibodies**. For example, a side [sic] range of functional recombinant-antibody fragments, such as Fab, F_v, single-chain and single-domain antibodies, may be generated.

This passage indicates that recombinant expression makes possible the production of a variety of antigen-specific immunoglobulins including those known from proteolytic processing (e.g., Fab) and those known only by recombinant expression of light and heavy chain variable regions (e.g., single chain antibodies).

The ordinary skilled artisan would have appreciated that recombinant DNA methods can be used to produce any of a variety of antibody fragments and not just those known previously by proteolytic cleavage . This is evidenced by the state of the art as of the earliest filing date of the instant application. For example, U.S. Patent no. 4,816,567 to Cabilly et al., filed April 8, 1983, describes the use of recombinant DNA technology to express antibodies that have less than a full length heavy or light chain (Summary of the Invention; emphasis added).

> The invention relates to antibodies and to non-specific immunoglobulins (NSIs) formed by recombinant techniques using suitable host cell cultures. . . .
>
> Finally, either the light chain or heavy chain alone, **or portions thereof**, produced by recombinant techniques are included in the invention and may be mammalian or chimeric.

Cabilly also teaches recombinant expression of any and all immunologically active fragments by referring to expressing "at least the variable domain" of light and heavy chains.

U.S. Patent No. 4,704,692 to Ladner (cited on page 28 lines 5-13 of the instant application) teaches that recombinant methods can be used to express unique fragments of immunoglobulins in which terminal amino acids at the N- or C-terminus of the variable region of light or heavy chains are removed as part of the strategy for linking the chains with a peptide linker to form a single chain Fv fragment. Such antibody fragments would be immunologically active while comprising less than a full length variable domain and no constant domains.

Schwartzbaum et al. (Eur. J. Immunol., vol. 19(60, 1015-1023; 1989; attached as Exhibit A) used molecular biology techniques to construct IgE antibodies with deletions in either the $C_\varepsilon 4$ and $C_\varepsilon 3$ constants domains (see abstract). Similarly, Bettler et al. (PNAS, 86:7118-7122, 1989; attached as Exhibit B) describes preparation of a large number of IgE constant domain deletion mutants (see Fig. 2).[2]

Thus, it is respectfully submitted that the phrase "portion thereof," added previously to claims 43 and 83, is amply supported by the specification and, therefore, raises no issue of new matter.

REJECTION UNDER 35 U.S.C. § 102 OVER DÜRING

The rejection of claims 21, 32-39, 42-47, 49-54, 56, 57, 60-66, 68, 70-75, 78, and 80-82 under 35 U.S.C. § 102(b) as being allegedly anticipated by the During

[2] These mutants were expressed as Fc fragments.

dissertation is respectfully traversed. The Examiner is referred to the relevant sections of the Response filed March 14, 2002 and the Amendment filed March 4, 2002 for Applicants' response. With respect to claim 83 as now amended, it is further noted that During never attempted nor described expression in plants of heavy chains without light chains. As discussed extensively in the earlier cited responses, During was never even able to detect the presence of heavy chains by Western blotting or by immunogold electron microscopy even when he attempted to express a heavy chain simultaneously with a light chain. In view of these arguments, it is respectfully submitted that there is no basis to find the claims anticipated by the During dissertation.

REJECTION UNDER 35 U.S.C. § 102 OVER GOODMAN

The rejection of claims 21, 32-40, 42-47, 49-54, 56-58, 60-66, 68, 70-76, and 78-82 under 35 U.S.C. § 102(e) as being allegedly anticipated by Goodman (U.S. No. 4,956,282) is respectfully traversed. The Examiner is referred to the relevant sections of the Response filed March 14, 2002 and the Amendment filed March 4, 2002 for Applicants' response. In short, Goodman makes no mention whatsoever of any of the immunoglobulin forms presently claimed. In view of these arguments, it is respectfully submitted that there is no basis to find the claims anticipated by Goodman.

REJECTION UNDER 35 U.S.C. § 103 OVER DÜRING

The rejection of claims 21, 32-54, 56-66 and 68-82 under 35 U.S.C. § 103(a) as being allegedly unpatentable over Düring in view of Applicant's allegedly admitted prior art is respectively traversed. The Examiner is referred to the relevant sections of the Response filed March 14, 2002 and the Amendment filed March 4, 2002 for Applicants' response. As mentioned above, During was never even able to detect the presence of heavy chains by Western blotting or by immunogold electron microscopy even when he attempted to express a heavy chain simultaneously with a light chain. In view of these arguments, it is respectfully submitted that there is no basis to find the claims obvious over the During dissertation.

Atty. Dkt. No. EPI3007B
(formerly TSRI 184.2C2)

REJECTION UNDER 35 U.S.C. § 103 OVER GOODMAN

The rejection of claims 21, 32-54, 56-66 and 68-82 under 35 U.S.C. § 103(a) as being allegedly unpatentable over Goodman in view of Applicant's allegedly admitted prior art is respectively traversed. The Examiner is referred to the relevant sections of the Response filed March 14, 2002 and the Amendment filed March 4, 2002 for Applicants' response.

Claim 43 and its dependents are not obvious over Goodman

In addition to the arguments already of record, it is further noted that Goodman fails to even mention the requirement for a plant comprising plant cells containing nucleic acid encoding an immunoglobulin single polypeptide product, let alone a single polypeptide that comprises an immunoglobulin heavy chain polypeptide or portion thereof and an immunoglobulin light chain or portion thereof and a peptide linker therebetween Goodman's passing reference to an immunoglobulins is does not come close to describing or meeting the requirements of an enabling disclosure.

It is further noted that Goodman's teachings on gamma interferon expression would not reasonably have been considered to advance the possibility of immunoglobulin expression in plants. Gamma interferon is naturally a single polypeptide while the instantly claimed single polypeptide immunoglobulin is an artificial combination of two chains which are separately expressed in the natural state. Furthermore, the instantly claimed single polypeptide immunoglobulin includes a peptide linker which is also lacking in the natural state. These distinctions over Goodman are above and beyond those already of record, which include the fact that gamma interferon is structurally and functionally distinct from immunoglobulin light or heavy chains, the latter of which are immunoglobulin superfamily members. Moreover, immunoglobulins have unique biology unto themselves such as the phenomenon known as heavy chain toxicity. Thus, the teachings of Goodman are largely irrelevant to the claim 43 and its dependents. Accordingly, in view of above prior arguments of record, the examiner is respectfully urged to withdraw the rejection of claims 43 and its dependent claims as allegedly obvious over Goodman.

Atty. Dkt. No. EPI3007B
(formerly TSRI 184.2C2)

Claim 83 and its Dependents are not obvious over Goodman

In addition to the arguments already of record, it is further noted that Goodman fails to even mention the requirement for a plant comprising plant cells containing nucleic acid encoding an immunoglobulin heavy chain. Goodman does not even enable expression of a dual chain immunoglobulin, which it mentions only in passing, let alone enabling and rendering obvious plant expression of a heavy chain polypeptide in a manner that allows it to form an antigen-specific immunoglobulin when co-expressed with the light chain from antigen-specific immunoglobulin from which the heavy chain was obtained. Goodman's success with gamma interferon is of no significance to the claimed invention because although gamma interferon is a single polypeptide, it is naturally a single polypeptide. Expressing a natural single polypeptide does not predict whether one can successfully express a single polypeptide from two chains that are naturally separately expressed and assemble to form a heterodimer. This is so particularly true in the case of immunoglobulins which have unique biology such as the phenomenon known as heavy chain toxicity. Accordingly, in view of the above and prior arguments of record, the Examiner is respectfully requested to withdraw the rejection of claims 83 and its dependent claims as allegedly obvious over Goodman.

Applicant believes that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is urged to contact the undersigned by telephone to address any outstanding issues standing in the way of an allowance.

Respectfully submitted,

Date: May 30, 2002

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

43 (Three times amended) A plant, comprising plant cells containing:

a) nucleotide sequences encoding an antigen-specific immunoglobulin single polypeptide product containing at least an immunoglobulin heavy chain polypeptide or portion thereof and an immunoglobulin light chain or portion thereof **and encoding a peptide linker therebetween**, wherein said nucleotide sequences encode a leader sequence forming a secretion signal for said single polypeptide product; and

b) antigen-specific immunoglobulin single polypeptide product encoded by said nucleotide sequences, wherein said leader sequence is cleaved from said polypeptide product following proteolytic processing and assembly.

83. (Amended) A plant, comprising plant cells containing:

a) nucleotide sequence encoding an immunoglobulin single polypeptide product containing an immunoglobulin heavy chain polypeptide [~~or an immunoglobulin light chain but not both~~], wherein said nucleotide sequence encodes a leader sequence forming a secretion signal for said single polypeptide product, said heavy chain [~~or light chain are~~] **derived** from an antigen-specific immunoglobulin **comprising a heavy and light chain**, and said single polypeptide product being capable of forming an antigen-specific immunoglobulin when co-expressed in the same cell with [~~the other of~~] said **light** chain from said antigen-specific immunoglobulin; and

b) immunoglobulin single polypeptide product encoded by said nucleotide sequences, wherein said leader sequence is cleaved from said polypeptide product following proteolytic processing of said single polypeptide product.